## Searching for a Potential Anticancer Drug. Design of Anti-anigiogenic Compounds Based on the Structure of Human Methionine Aminopeptidase.

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Beamline(s): **X12C, X8C**

**Introduction**: Angiogenesis, the formation of new blood vessels, is essential for tumor growth. Inhibition of angiogenesis is therefore emerging as a promising therapy for cancer. The present project aims at the design of anti-angiogenic compounds based on the structure of human methionine aminopeptidase 2 ( MetAP-2), a metalloprotease involved in endothelial cell proliferation and neovascularization. The natural products fumagillin and ovalicin and various synthetic analogues all inhibit angiogenesis through the specific cellular target MetAP-2. The crystallographic structure of MetAP-2 is being used as a basis for modeling a reversible inhibitor. Several substrate-based molecules were synthesized in our lab and are currently being tested as potential inhibitors. The structure of MetAP-2 in complex with that new inhibitor may provide information to elucidate the molecular mechanism of MetAP-2 as well as new insights for the design of a selective inhibitor.

**Methods and Materials**: Crystals of native MetAP-2 are obtained as described (Liu *et al*., 1998). Crystals are transferred to a stabilizing solution where a 10-fold excess of inhibitor is added. Soaking under different soaking times were tested.

Crystals were collected at Beam Lines X12C and X8C. A total of 12 complete data sets were obtained with maximum resolution ranging from 1.7 to 2.5Å.

**Results**: Fourier difference maps were calculated for all 12 different data sets. The electron density indicates that those inhibitors either bound with low occupancy or did not bind at all; even tough enzymatic assays suggested that those molecules inhibit MetAP-2 at range of nano molar concentrations. The extra electron density observed for some of the data sets were associated with the presence of the inhibitors at low occupancies and were not clear enough to model their conformation at the active site. No further refinement was performed. Analysis of the electron density map for the complete structure was performed and no other site of extra electron density was identified.

**Conclusions**: The structures where electron density could be associated with the presence of the inhibitors at low occupancy where not clear enough to model the conformation of those, but indicated that this class of inhibitors interact with the metals located in the active site. The ions are shown to bind with very low occupancy and we associate the unsuccessful results with the lack of ions. We believe that the buffer during the crystallization process is rescuing the ions and new experiments where an excess of metals will be added before soaking the inhibitors are in progress.

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**References**: Liu, S., Widon, J., Kemp, C.W., Crews, C.M. and Clardy, J. (1998) *Science* **282**, 1324-1327.